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Published in:
British Journal of Nutrition

DOI:
10.1017/S0007114510003405

Publication date:
2011

British Journal of Nutrition (2011), **105**, 268–274
doi:10.1017/S0007114510003405

The effect of enteral supplementation of a prebiotic mixture of non-human milk galacto-, fructo- and acidic oligosaccharides on intestinal permeability in preterm infants

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(Received 18 January 2010 – Revised 16 July 2010 – Accepted 20 July 2010 – First published online 24 September 2010)

Abstract

Preterm infants have an impaired gut barrier function. We aimed to determine the effects of enteral supplementation of a prebiotic mixture consisting of neutral oligosaccharides (short-chain galacto-oligosaccharides ($_{SC}$GOS)/long-chain fructo-oligosaccharides ($_{LC}$FOS)) and acidic oligosaccharides (AOS) on intestinal permeability of preterm infants as measured by the sugar absorption test in the first week of life. Furthermore, we determined host- and treatment-related factors associated with intestinal permeability. In a randomised controlled trial, preterm infants with a gestational age <32 weeks and/or birth weight (BW) <1500 g received enteral supplementation of $_{SC}$GOS/$_{LC}$FOS/AOS or placebo (maltodextrin) between days 3 and 30 of life. Intestinal permeability, reflected by the urinary lactulose/mannitol (L/M) ratio after oral ingestion of lactulose and mannitol, was assessed at three time points: before the start of the study ($t = 0$), at day 4 ($t = 1$) and at day 7 ($t = 2$) of life. Data were analysed by generalised estimating equations. In total, 113 infants were included. Baseline patient and nutritional characteristics were not different between the $_{SC}$GOS/$_{LC}$FOS/AOS (n 55) and the placebo groups (n 58). $_{SC}$GOS/$_{LC}$FOS/AOS had no effect on the L/M ratio between $t = 0$ and $t = 2$. In both the groups, the L/M ratio decreased from $t = 0$ to $t = 2$ ($P<0{\cdot}001$). Low BW increased the L/M ratio ($P=0{\cdot}002$). Exclusive breast milk feeding and mixed breast milk/formula feeding during the first week of life decreased the L/M ratio ($P<0{\cdot}001$ and $P<0{\cdot}05$, respectively). In conclusion, enteral supplementation of a prebiotic mixture does not enhance the postnatal decrease in intestinal permeability in preterm infants in the first week of life.

Key words: Preterm infants: Intestinal permeability: Prebiotics

Directly after birth, preterm infants have an impaired gut barrier function, reflected by an increased intestinal permeability[1,2]. Due to this increased intestinal permeability, delayed intestinal colonisation and immaturity of the host immune defence system, potentially pathogenic bacteria may translocate from the intestinal lumen and cause systemic infections[3–5]. Early enteral feeding is associated with decreased intestinal permeability[6]. In a recent study, intestinal permeability was decreased in preterm infants who received breast milk feeding *v.* preterm infants who received formula feeding[7]. These beneficial effects of breast milk may partially be attributed to the 'bifidogenic' effect of human milk. Increasing the number of 'bifidogenic' bacteria may improve gut barrier function and prevent systemic infections from translocation of gut bacteria[8,9]. Non-human milk oligosaccharides such as short-chain galacto-oligosaccharides ($_{SC}$GOS) and long-chain fructo-oligosaccharides ($_{LC}$FOS) have been developed[10]. Non-human milk acidic oligosaccharides (AOS) can be derived from pectin. In studies with non-human milk oligosaccharides, enteral supplementation of neutral oligosaccharides stimulates the growth of bifidobacteria and lactobacilli, resulting in increased SCFA production[10]. In an *in vitro* model, SCFA were able to stimulate mucin-2 production and improve gut barrier function[11]. In an experimental animal study, a diet containing a prebiotic mixture of inulin and oligofructose had a trophic effect on colonic mucosal architecture[12]. In addition, translocation

Abbreviations: AOS, acidic oligosaccharides; BW, birth weight; GA, gestational age; $_{LC}$FOS, long-chain fructo-oligosaccharides; L/M, lactulose/mannitol; NICU, neonatal intensive care unit; SAT, sugar absorption test; $_{SC}$GOS, short-chain galacto-oligosaccharides.

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of *Salmonella typhimurium* after oral inoculation was partly decreased by the prebiotic diet[13], suggesting a beneficial effect on gut barrier function.

In the initial study, we found a trend towards a decreased incidence of serious endogenous infections after enteral supplementation of a prebiotic mixture consisting of neutral oligosaccharides ($_{SC}$GOS/$_{LC}$FOS) and AOS, if given in sufficient amounts[14]. We hypothesised that the lower endogenous infection rate in preterm infants receiving $_{SC}$GOS/$_{LC}$FOS/AOS may originate from an improved gut barrier function.

Until now, studies into the effect of prebiotics on intestinal permeability in adults and newborn infants showed controversial results[9,15–19]. In preterm infants, intestinal permeability was found to decrease spontaneously in the first week of life[1,2,19,20]. We hypothesise that enteral supplementation of a prebiotic mixture consisting of neutral oligosaccharides ($_{SC}$GOS/$_{LC}$FOS) and AOS may improve gut barrier function, especially in the first week of life, as reflected by decreased intestinal permeability. Therefore, the aim of the present study was to determine the effect of enteral supplementation of $_{SC}$GOS/$_{LC}$FOS/AOS on intestinal permeability as measured with the sugar absorption test (SAT) in the first week of life in preterm infants. Furthermore, we determined host- and treatment-related factors associated with intestinal permeability in these preterm infants.

Experimental methods

Subjects

Infants with a gestational age (GA) <32 weeks and/or birth weight (BW) <1500 g admitted to the level III neonatal intensive care unit (NICU) of the VU University Medical Center, Amsterdam were eligible for participation in the study. Exclusion criteria were small-for-GA infants with a GA >34 weeks, major congenital or chromosomal anomalies, death <48 h after birth and transfer to another hospital <48 h after birth. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human patients were approved by the medical ethical review board of our hospital. Written informed consent was obtained from all the parents.

Randomisation, blinding and treatment

After assignment to one of three BW groups ($\leq$799, 800–1199 and $\geq$1200 g), infants were randomly allocated <48 h after birth to receive either an enteral supplementation of a prebiotic mixture of 80 % $_{SC}$GOS/$_{LC}$FOS and 20 % AOS or a placebo mixture of maltodextrin. An independent researcher used a computer-generated randomisation table (provided by Danone Research, Friedrichsdorf, Germany) to assign infants to treatment with $_{SC}$GOS/$_{LC}$ FOS/AOS or placebo. Investigators, parents, medical and nursing staff were unaware of treatment allocation. The randomisation code was broken after complete data analysis had been performed.

$_{SC}$GOS/$_{LC}$FOS/AOS and the placebo (maltodextrin) were prepared and packed sterile (Danone Research). The two powders were indistinguishable by appearance, colour and smell. During the study period, $_{SC}$GOS/$_{LC}$FOS/AOS and placebo were monitored for stability and possible microbiological contamination.

The supplementation of $_{SC}$GOS/$_{LC}$FOS/AOS or placebo was administered in increasing doses between days 3 and 30 of life to a maximum of 1·5 g/kg per d to breast milk or preterm formula (Nenatal Start®). Due to osmolarity reasons, each infant had an individual feeding scheme depending on BW and daily amount of feeding. If an infant received $\leq$100 ml/kg per d enteral feeding, 1 g $_{SC}$GOS/$_{LC}$FOS/AOS or placebo was added per 60 ml enteral feeding. If an infant received $>$100 ml/kg per d, 1 g $_{SC}$GOS/$_{LC}$FOS/AOS or placebo was added per 100 ml enteral feeding. Two members of the nursing staff added the daily supplementation to breast milk or to preterm formula according to the parents' choice. Per 100 ml, the preterm formula provided 350 kJ (80 kcal), 2·4 g protein (casein-to-whey protein ratio 40:60), 4·4 g fat and 7·8 g carbohydrate. The preterm formula did not contain oligosaccharides. When infants were transferred to another hospital before the end of the study, the protocol was continued under supervision of the principal investigator (E. A. M. W.).

Nutritional support

Protocol guidelines for the introduction of parenteral and enteral nutrition followed current practice at our NICU. Nutritional support was administered as previously described[21], except for minimal enteral feeding which was defined as 12–24 ml/kg per d. Enteral nutrition was advanced either from day 2 or from day 4 in case of a BW $<$10th percentile, GA <26 weeks, Apgar score <6 at 5 min, umbilical artery pH $<$7·10 or base deficit $>$10 mmol/l. For each infant in the study, a feeding schedule was proposed based on BW and the guidelines as mentioned previously. After discharge, all the infants received breast milk or preterm formula (Nenatal Start® without oligosaccharides) until term, and a post-discharge formula (Nenatal 1® without oligosaccharides) until the corrected age of 6 months. The medical staff of our NICU and the responsible paediatricians in the regional hospitals had final responsibility for the administration of parenteral nutrition and advancement of enteral nutrition. Further details on the initial study have previously been published[14,22].

Intestinal permeability

Intestinal permeability was measured by SAT, as previously described[2], at three time points: before the start of the

study ($t = 0$), at day 4 ($t = 1$), and at day 7 ($t = 2$) after birth. In the SAT, urine was collected for 6 h after instillation of 2 ml/kg of the test solution (100 mg mannitol and 250 lactulose/5 ml sterile water) by nasogastric tube. After collection, 0·1 ml chlorhexidine digluconate (20 %) was added to the urine as a preservative, and samples were stored at −20°C until analysis. Lactulose and mannitol were measured by GC, and the lactulose/mannitol (L/M) ratio was calculated.

Nutrition and clinical characteristics of the infants at the time of SAT were assessed, including administration of type of feeding in the week preceding the SAT, parenteral nutrition, achievement of full enteral feeding and presence of serious infection(s). A serious infection was defined as sepsis, meningitis, pyelonephritis, pneumonia or arthritis as diagnosed by a combination of clinical signs and a positive culture[23] within 48 h preceding the SAT.

Statistical analysis

The sample size of 113 infants was based on the sample size calculation for the primary outcome of the main trial (serious infectious morbidity). Normally distributed and non-parametric data are presented as mean (SD) and medians (ranges), respectively. Perinatal and nutritional characteristics were analysed by Student's t test, Mann–Whitney U test, χ^2 test or Fisher's exact test for continuous normally distributed, non-parametric continuous and dichotomous data, respectively.

As the parameters of intestinal permeability had a skewed distribution, a natural logarithmic transformation was performed before analysis. In the primary analysis, generalised estimating equations for longitudinal analysis were used to compare changes in lactulose, mannitol and L/M ratio over time between the groups[24]. This method takes into account the dependency of the observations within a patient and the fact that samples may not be available at each time point. Furthermore, the effect of host- and treatment-related factors (chorioamnionitis, administration of antenatal corticosteroids, mode of delivery, GA, BW, Apgar score at 5 min, administration of antibiotics postpartum, serious infectious morbidity, necrotising enterocolitis[25], time to full enteral feeding (>120 ml/kg per d), age at finishing parenteral nutrition and type of feeding during the first week of life on intestinal permeability) was determined by generalised estimating equation analysis. All the statistical analysis was performed on an intention-to-treat basis. For all the statistical analyses, a two-sided P value <0·05 was considered significant. SPSS 15.0 (SPSS, Inc., Chicago, IL, USA) was used for data analysis.

Results

Between May 2007 and November 2008, 113 of 208 eligible preterm infants entered the study. Reasons for not participating in the study were no informed consent (n 45), participation in another trial (n 7), transfer to a regional hospital within 48 h (n 12), death within 48 h (n 5) and severe congenital malformations (n 12). After randomisation, one infant in the placebo group was excluded, because of strong suspicion of a syndrome. Baseline patient and nutritional characteristics were not different in $_{SC}$GOS/$_{LC}$FOS/AOS (n 55) and placebo groups (n 58)[14] (Table 1). SAT was performed at 36 (SD 15) h after birth ($t = 0$), at postnatal day 4·5 (SD 0·7) ($t = 1$) and at postnatal day 7·1 (SD 0·5) ($t = 2$). At $t = 0$, 17/55 (30·9 %) and 10/58 (17·1 %), at $t = 1$, 20/55 (36·4 %) and 11/58 (19·0 %), and at $t = 2$, 15/55 (27·3 %) and 15/58 (25·9 %) of the SAT data were missing in the $_{SC}$GOS/$_{LC}$FOS/AOS and placebo groups, respectively. Missing SAT data could mainly be attributed to insufficient urine collections. In both groups, the L/M ratio showed a decrease from $t = 0$ (0·21 (0·03–2·16) *v.* 0·34 (0·06–1·86) in $_{SC}$GOS/$_{LC}$FOS/AOS-supplemented and placebo groups, respectively, to $t = 2$ (0·06 (0·00–0·68) *v.* 0·09 (0·00–2·68)) in $_{SC}$GOS/$_{LC}$FOS/AOS-supplemented and placebo groups, respectively (effect 0·34 (95 % CI 0·22, 0·51; $P<0·001$) (Fig. 1(a)). Analysis by generalised estimating equations showed no effect of enteral supplementation of $_{SC}$GOS/$_{LC}$FOS/AOS on the decrease in L/M ratio. Lactulose and mannitol concentrations were not different in the $_{SC}$GOS/$_{LC}$FOS/AOS-supplemented and placebo groups (Fig. 1(b) and (c)).

As there were no significant differences between the $_{SC}$GOS/$_{LC}$FOS/AOS-supplemented and placebo groups, both the groups were analysed together to determine the influence of different host- and treatment-related factors on intestinal permeability (Table 2). Increased BW was related to decreased intestinal permeability (effect 0·54 (95 % CI 0·36, 0·81; $P=0·002$)). Both exclusively breast milk feeding and mixed breast milk/formula feeding in the first week of life decreased the L/M ratio compared with exclusively formula feeding (effect 0·49 (95 % CI 0·34, 0·73; $P<0·001$) and 0·53 (95 % CI 0·30, 0·93; $P<0·05$), respectively). At $t = 2$, 22/113 (20 %) of the infants had a serious infection. SAT was performed in 16/22 (73 %) of the infants with a serious infection. The median L/M ratio was not different in infants with a serious infection and infants without a serious infection. In total, sixteen infants developed necrotising enterocolitis. The median age at which infants developed necrotising enterocolitis was 21 d (6–60). The median L/M ratio was not different in infants who later developed necrotising enterocolitis compared to infants who did not develop necrotising enterocolitis (Table 2).

Discussion

In preterm infants, we found that enteral supplementation of a prebiotic mixture consisting of neutral oligosaccharides ($_{SC}$GOS/$_{LC}$FOS) and acidic (AOS) oligosaccharides does not decrease intestinal permeability in the first week of life, as measured by the SAT. The present results are in line with a study in healthy newborns[18]. Also, in

Table 1. Baseline and nutritional characteristics*
(Percentage values, standard deviations and ranges)

	Prebiotic mixture (*n* 55)		Placebo (*n* 58)	
Variable†	Infants (%)	SD/range	Infants (%)	SD/range
Baseline characteristics				
Chorioamnionitis	20		22	
PE, E or HELLP	31		31	
Placental insufficiency	7		5	
Antenatal antibiotics	20		28	
Antenatal corticosteroids (%)	56		56	
Multiple birth	16		22	
Vaginal delivery (%)	56		55	
Gestational age (weeks)	29·9	1·9	29·3	2·1
Birth wt (kg)	1·3	0·4	1·2	0·3
Birth wt < 10th percentile‡	22		14	
Sex (% male)	56		62	
Apgar score at 5 min < 6	16		9	
Antibiotics postpartum	75		76	
Nutritional characteristics				
Age at the start of study supplementation (d)	2·1	1·5–5·3	2·1	1·5–3·3
Time to full supplementation dose (d)	11	4–28	11	5–27
Mean supplementation dose during the study period (g/kg per d)	1·30	0·1–1·6	1·27	0·2–1·8
Age at introduction of enteral nutrition (d)	2·8	0·6–27·5	2·5	0·3–18·0
Exclusive breast milk during the 30 d study period (%)	69		57	

PE, pre-eclampsia; E, eclampsia; HELLP, syndrome of haemolysis, elevated liver enzymes and low platelets.
* Baseline and nutritional characteristics were not statistically different ($P<0·05$) between the prebiotic mixture and placebo group.
† Student's *t* test, Mann–Whitney *U* test and χ^2 test or Fisher's exact test are used to analyse continuous normally distributed, non-parametric continuous data, respectively.
‡ According to Usher & McLean[39].

adult burn patients, prebiotic supplementation did not decrease intestinal permeability[15].

The lack of effect of prebiotics on intestinal permeability may be explained by high doses of antibiotics used in patients, including preterm infants, admitted at a intensive care unit, which interferes with the growth of bifidobacteria and lactobacilli[15]. In two recent studies in preterm infants, we found that antibiotics severely delayed the intestinal colonisation[26,27]. Delayed intestinal colonisation decreases the production of SCFA which in turn impairs gut barrier function[10,11]. In the present study, 75% of all infants received antibiotics immediately after birth. Furthermore, the prebiotic supplementation dose in the present study may have been insufficient to reach an (maximal) effect on intestinal permeability. This is in accordance with the trend towards a lower incidence of endogenous infections if preterm infants received prebiotics in a sufficient amount and numbers of days[14]. In the literature, few data are available about the optimal type, combination and amount of prebiotic supplementation[28]. Olguin *et al.*[15] used one type of prebiotics (oligofructose), and Colomé *et al.*[18] did not state which type and amount of prebiotics were given. Furthermore, probiotics and, especially, the combination of pre- and probiotics could be used, because a synergistic or additional effect may exist[28]. Stratiki *et al.*[19] found that administration of a probiotic (*Bifidobacterium lactis*)-supplemented formula decreased intestinal permeability of preterm infants at day 30. In adult trauma patients, synbiotic, but not prebiotic (fermentable fibres), supplementation decreased intestinal permeability[16].

The timing of the prebiotic supplementation may also explain the varying effects on intestinal permeability. The mean age of infants in the study of Colomé *et al.*[18] was 74·4 (SD 30·3) d which could explain the lack of effect on intestinal permeability of the prebiotic supplementation, as previous studies show that intestinal permeability decreases rapidly in the first week of life[1,2]. Stratiki *et al.*[19] found that probiotics decreased intestinal permeability at day 30 of life, but not at day 7. This finding suggests that supplementation of prebiotics, probiotics or synbiotics may affect intestinal permeability, if given for a sufficient number of days.

Postnatal factors may play an important role in the rapid adaptation of the small intestine to the extrauterine circumstances[20]. In the present study, we found that both exclusively breast milk feeding and mixed breast milk/formula feeding during the first week of life decreased intestinal permeability at day 7. This is in line with a recent study done by Taylor *et al.*[7] who found a decreased intestinal permeability in preterm breast-fed infants during the first 30 d of life. A decreased intestinal permeability after breast milk feeding was also found in term infants[6,29]. This positive effect of breast milk feeding may be attributed to the 'bifidogenic' effect of breast milk and supports the hypothesis that the intestinal microbiota plays an important role in gut barrier function. The intestinal microbiota communicates with the underlying epithelium, which may lead to metabolic and immunologic reactions by the epithelial cells and its underlying lymphoid cells. This process is called bacterial–epithelial 'crosstalk'[30,31]. Preterm infants have an inadequate maturation of the

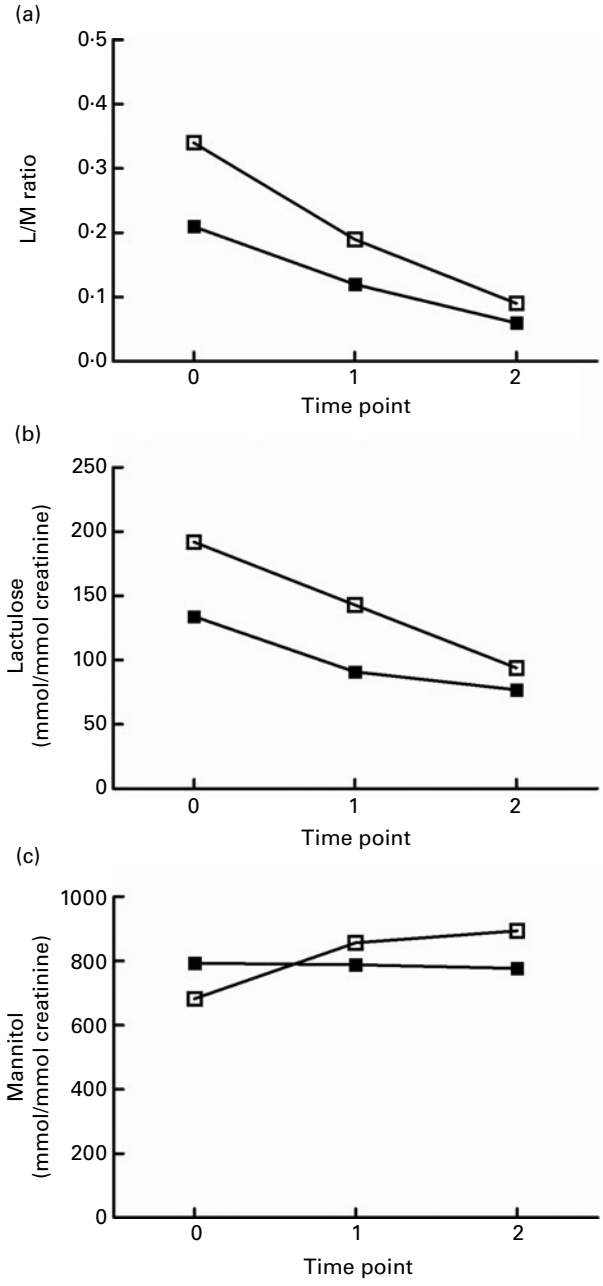

Fig. 1. Results of sugar absorption tests. Results are expressed as median. Time points: before the start of the study ($t = 0$), at day 4 ($t = 1$) and at day 7 ($t = 2$) after birth. (a) Urinary lactulose/mannitol (L/M) ratio. (b) Urinary lactulose concentrations. (c) Urinary mannitol concentrations. ■, Prebiotic mixture; □, placebo. There were no significant differences between both the groups (generalised estimated equations).

host immune defence system, and due to an inappropriate bacterial–epithelial 'crosstalk'[31], they have an increased risk to develop serious infections. However, the decreased incidence of endogenous infections[14] could not be explained by improved gut barrier function, as reflected by intestinal permeability.

Besides a high risk for serious infectious morbidity, preterm infants are at high risk for developing necrotising enterocolitis. The pathogenesis of necrotising enterocolitis

Table 2. Factors influencing intestinal permeability (lactulose/mannitol (L/M) ratio) in preterm infants

(Effects and 95 % confidence intervals)

	Effect†	95 % CI
Chorioamnionitis	0·93	0·65, 1·32
Antenatal corticosteroids	1·01	0·75, 1·37
Vaginal delivery	0·96	0·72, 1·31
Gestational age (weeks)	0·95	0·88, 1·02
Birth wt (kg)	0·54**	0·36, 0·81
Apgar score at 5 min <6	0·91	0·59, 1·40
Antibiotics postpartum	1·02	0·46, 1·39
Infection <48 h before sample at day 7‡	0·87	0·53, 1·42
Necrotising enterocolitis	1·22	0·76, 1·97
Time to full enteral feeding	1·01	0·98, 1·03
Age at finishing parenteral nutrition	1·00	0·98, 1·04
Exclusive breast milk in the firstweek of life§	0·49***	0·34, 0·73
Mixed breast milk and formula feeding in the first week of life§	0·53*	0·30, 0·93

The factor significantly influenced intestinal permeability: *$P<0·05$, **$P<0·01$, ***$P<0·001$.

† Data indicate the effect of a factor on the L/M ratio at all time points (generalised estimated equations). The effect can be interpreted as follows: in case of chorioamnionitis, the L/M ratio is 0·93 (95 % CI) times as high as without chorioamnionitis.

‡ Sepsis, meningitis, pyelonephritis, pneumonia or arthritis as diagnosed by a combination of clinical signs and a positive culture <48 h preceding sugar absorption test.

§ Compared with exclusive formula feeding.

is not completely understood, but it has been proposed that impaired gut barrier function plays a crucial role[32]. In a rat gavage model, Zani *et al.*[33] found that rats with experimentally induced necrotising enterocolitis have increased intestinal permeability and develop systemic symptoms such as cardiac damage and renal failure. This suggests bacterial translocation and transfer of endotoxin and other inflammatory mediators causing multi-organ failure[33]. In the present study, we did not find an increased intestinal permeability in infants who developed necrotising enterocolitis. However, in most cases, the SAT was performed before the infants developed necrotising enterocolitis.

Some remarks may be formulated with regard to the methodology of the present study. First, we only measured intestinal permeability in the first week of life. Study supplementation started at a median postnatal age of 2 d. At postnatal day 7, 86/113 (76 %) of the infants had not yet reached a supplementation dose of 1·5 g/kg per d. The mean supplementation dose during the first week of life was 0·73 (SD 0·43) g/kg per d. Therefore, infants may not have received a sufficient dose of $_{SC}$GOS/$_{LC}$FOS/AOS supplementation to reach a significant effect at postnatal day 4 or 7. However, in an additional analysis, we did not find a relationship between supplementation dose during the first week of life and intestinal permeability. Furthermore, in a previous study, in the first week of life, a significant decrease of the intestinal permeability was found, and during the next 3 weeks, the intestinal permeability remained stable[21]. Therefore, we hypothesised

that the maximum effect of prebiotic supplementation on intestinal permeability would be in the first week of life. In the present study, intestinal permeability decreased in both the groups from day 1 to day 7. This is in line with previous studies[1,2,19,20]. Secondly, breast milk itself contains neutral and AOS and, as shown in the present study, breast milk feeding has a positive effect on intestinal permeability. Therefore, the effect of enteral supplementation of $_{SC}$GOS/$_{LC}$FOS/AOS may be less pronounced in preterm infants who exclusively received breast milk. As breast milk is strongly promoted at our NICU, most infants received breast milk feeding (>60 %), and relatively few received exclusively formula feeding (20 %).

There were no serious adverse events reported after prebiotic supplementation in two recent reviews on prebiotic supplementation, including preterm infants[34,35]. However, Barrat *et al.*[36] found an increased bacterial translocation in the intestine of immature rats ('pup in the cup model') fed a milk formula containing GOS and inulin, without an effect on intestinal permeability. However, intestinal permeability was measured *in vitro* with Ussing chambers[36]. The mechanisms underlying this increased bacterial translocation are unclear and should be investigated further. Bacterial translocation may originate from a combination of increased intestinal permeability, delayed intestinal colonisation and immaturity of the host immune defence[3,5]. It has been speculated that the increased bacterial translocation may not necessarily be harmful, but may be involved in the postnatal maturation of the immune system[37,38]. Urao *et al.*[37] and Gebbers & Laissue[38] speculate that bacterial translocation may be instrumental for tolerance induction against the endogenous microbiota and for the stimulation and normal development of the gut-associated lymphoid tissue.

In conclusion, the present study in preterm infants shows that enteral supplementation of a prebiotic mixture consisting of neutral ($_{SC}$GOS/$_{LC}$FOS) and acidic (AOS) oligosaccharides does not enhance the decrease in intestinal permeability in the first week of life, as measured by the SAT. Breast milk feeding during the first week of life decreased the L/M ratio. The trend towards a lower incidence of endogenous infection rate in preterm infants receiving $_{SC}$GOS/$_{LC}$FOS/AOS cannot be explained by improved gut barrier function, as reflected by intestinal permeability in the first week of life. A beneficial effect of $_{SC}$GOS/$_{LC}$FOS/AOS may involve other aspects of gut barrier function; for example, modulation of the intestinal microbiota and the intestinal inflammatory response.

Acknowledgements

Study supplementation ($_{SC}$GOS/$_{LC}$FOS/AOS and maltodextrin) and preterm formula (Nenatal Start®) and post-discharge formula (Nenatal 1®) for the present study were provided by Danone Research. We acknowledge the parents for allowing their infants to participate in the study. Furthermore, we also thank the medical and nursing staff of the NICU of the VU University Medical Center and all participating hospitals and Henk Breukelman (Laboratory Center for Special Analysis, University Medical Center Groningen, The Netherlands) for analysing the urinary samples. The authors have no conflict of interest. The authors' contributions were as follows: E. A. M. W., H. N. L., W. P. F. F. and R. M. v. E. formulated the research questions and participated in the study design. E. A. M. W. and R. M. v. E. coordinated the study. E. A. M. W. and A. v. d. B. analysed the data. E. A. M. W. wrote the draft for the manuscript, and all the authors critically reviewed and revised the manuscript. The funding source had no involvement in the analysis of the data or the interpretation of the results. All the authors approved the final version of the manuscript. The study is registered at isrctn.org as ISRCTN16211826.

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